

## 4.5 PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Central Valley Steelhead Genetic Evaluation  
 Applicant Name: Dennis McEwan  
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Amount of funding requested: \$ \$70,636 for 2.5 years

Indicate the Topic for which you are applying (check only one box).

- |                                                      |                                                              |
|------------------------------------------------------|--------------------------------------------------------------|
| <input type="checkbox"/> Fish Passage/Fish Screens   | <input type="checkbox"/> Introduced Species                  |
| <input type="checkbox"/> Habitat Restoration         | <input checked="" type="checkbox"/> Fish Management/Hatchery |
| <input type="checkbox"/> Local Watershed Stewardship | <input type="checkbox"/> Environmental Education             |
| <input type="checkbox"/> Water Quality               |                                                              |

Does the proposal address a specified Focused Action? ☒ yes ☐ no

What county or counties is the project located in? Shasta, Tehama, Butte, Glenn, Colusa, Sutter Yuba, Placer, Yolo, Sacramento, Solano, San Joaquin, Calaveras, Stanislaus, Tuolumne, Merced

Indicate the geographic area of your proposal (check only one box):

- |                                                     |                                                                            |
|-----------------------------------------------------|----------------------------------------------------------------------------|
| <input type="checkbox"/> Sacramento River Mainstem  | <input type="checkbox"/> East Side Trib:                                   |
| <input type="checkbox"/> Sacramento Trib:           | <input type="checkbox"/> Suisun Marsh and Bay                              |
| <input type="checkbox"/> San Joaquin River Mainstem | <input type="checkbox"/> North Bay/South Bay:                              |
| <input type="checkbox"/> San Joaquin Trib:          | <input checked="" type="checkbox"/> Landscape (entire Bay-Delta watershed) |
| <input type="checkbox"/> Delta:                     | <input type="checkbox"/> Other:                                            |

Indicate the primary species which the proposal addresses (check all that apply):

- |                                                                                              |                                                     |
|----------------------------------------------------------------------------------------------|-----------------------------------------------------|
| <input type="checkbox"/> San Joaquin and East-side Delta tributaries fall-run chinook salmon | <input type="checkbox"/> Spring-run chinook salmon  |
| <input type="checkbox"/> Winter-run chinook salmon                                           | <input type="checkbox"/> Fall-run chinook salmon    |
| <input type="checkbox"/> Late-fall run chinook salmon                                        | <input type="checkbox"/> Longfin smelt              |
| <input type="checkbox"/> Delta smelt                                                         | <input checked="" type="checkbox"/> Steelhead trout |
| <input type="checkbox"/> Splittail                                                           | <input type="checkbox"/> Striped bass               |
| <input type="checkbox"/> Green sturgeon                                                      | <input type="checkbox"/> All chinook species        |
| <input type="checkbox"/> Migratory birds                                                     | <input type="checkbox"/> All anadromous salmonids   |
| <input type="checkbox"/> Other:                                                              |                                                     |

Specify the ERP strategic objective and target (s) that the project addresses. Include page numbers from the January 1999 version of ERP Volume I and II:

"determine the abundance, distribution, and structure of existing steelhead populations" - (ERPP vol I pg. 229, Feb 1999 version)

Indicate the type of applicant (check only one box):

|                                                          |                                         |
|----------------------------------------------------------|-----------------------------------------|
| <input checked="" type="checkbox"/> State agency         | <input type="checkbox"/> Federal agency |
| <input type="checkbox"/> Public/Non-profit joint venture | <input type="checkbox"/> Non-profit     |
| <input type="checkbox"/> Local government/district       | <input type="checkbox"/> Private party  |
| <input type="checkbox"/> University                      | <input type="checkbox"/> Other: _____   |

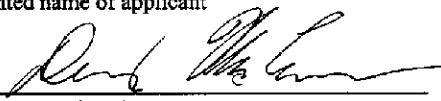
Indicate the type of project (check only one box):

|                                              |                                         |
|----------------------------------------------|-----------------------------------------|
| <input type="checkbox"/> Planning            | <input type="checkbox"/> Implementation |
| <input type="checkbox"/> Monitoring          | <input type="checkbox"/> Education      |
| <input checked="" type="checkbox"/> Research |                                         |

By signing below, the applicant declares the following:

- 1.) The truthfulness of all representations in their proposal;
- 2.) The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and
- 3.) The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

Dennis McEwan  
Printed name of applicant

  
Signature of applicant

# Central Valley Steelhead Genetic Evaluation

## **Applicant:**

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California Dept. of Fish and Game

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## **Participants and Collaborators**

California Department of Fish and Game  
California Department of Water Resources  
U.S. Geological Survey, Biological Resources Division  
U.S. Fish and Wildlife Service  
U.S. National Marine Fisheries Service

**Type of Organization:** State Agency

**Tax Identification Number:** 94-1697567

## Executive Summary

In response to the substantial decline of Central Valley steelhead, considerable planning efforts have begun recently to bring about recovery of steelhead and other anadromous fishes of the Central Valley aquatic ecosystem. However, a comprehensive baseline analysis of Central Valley steelhead genetics is lacking in these planning efforts. The National Marine Fisheries Service previously conducted genetic research on Central Valley steelhead populations, as part of a coastwide status review to delineate Evolutionarily Significant Units (ESU's). The broad scope of the study provided information that was useful for this purpose but did not have the resolution necessary to provide meaningful information within ESU's, such as the Central Valley. There is a need for a comprehensive genetic evaluation that will provide essential genetic information on Central Valley steelhead populations. The purpose of this project is to evaluate and describe the genetic and population structure and genetic variation of Central Valley steelhead populations by analyzing mtDNA and microsatellite DNA. A comprehensive genetic evaluation of Central Valley steelhead populations would provide needed information on the phylogenetic relationships among putative native rainbow trout/steelhead, naturally spawning steelhead, and hatchery steelhead. The foremost benefit of this evaluation will be to identify the most appropriate steelhead populations to use as donor stocks for reintroduction of steelhead to stream systems where they have become extirpated. Other benefits would be an assessment of whether hatchery practices are having unintended genetic effects on the natural populations and an elucidation of structure and genetic variation of natural populations. Collection of steelhead/rainbow trout tissue will be made from approximately 20 locations throughout the Sacramento/San Joaquin river system from July 1999 through August 2001. Tissue from 50 to 100 individuals will be collected from each site using standard methods of collection. Microsatellite DNA and mtDNA will be analyzed at the U.S. Geological Survey, Biological Resources Division Lab in Anchorage Alaska using standard techniques, and a final report and oral presentation of results and conclusions will be completed by April 2002. The project will cost \$174,258 with \$70,636 being requested from CALFED funds. The remainder of the funds will be provided as a cost share by the Department of Fish and Game and by CVPIA-AFRP. The Principal Investigators, Dennis McEwan and Dr. Jennifer Nielsen have extensive experience in steelhead biology, restoration, and genetics. This project is compatible with CALFED strategic objectives ("*determine the abundance, distribution, and structure of existing steelhead populations*"-ERPP vol. I), CMARP Draft Plan ("*A genetic evaluation of Central Valley steelhead populations is necessary to determine phylogenetic relationships among putative native rainbow trout, naturally spawning steelhead, and hatchery steelhead that were founded from non-native broodstock*"), and the CALFED 1999 Action Plan, which identifies steelhead as a high priority listed species.

## Project Description

**Background:** There has been a substantial decline in natural spawning populations of Central Valley steelhead, due primarily to loss of spawning and rearing habitat (McEwan and Jackson 1996). Yoshiyama et al. (1996) estimated that more than 82% of steelhead spawning and rearing habitat in the Central Valley has been lost.

The California Fish and Wildlife Plan (CDFG 1965) estimated that there were 40,000 adult steelhead in the Central Valley drainages in the late 1950's. Hallock et al. (1961) estimated the average annual steelhead run size was 20,540 adults in the Sacramento River system above the

mouth of the Feather River in the 1950's. Although an accurate estimate of the present population size is not available, a reliable indicator of the magnitude of the decline of Central Valley hatchery and wild stocks is the trend reflected in the Red Bluff Diversion Dam (RBDD) counts, which declined from an average annual count of 11,187 adults for the ten-year period beginning in 1967, to 2,202 adults annually in the early 1990's (McEwan and Jackson 1996). Natural spawning escapement estimates above RBDD for the period 1967 to 1991 averaged 3,465 and ranged from 0 (1989 and 1991) to 13,248 (1968) (McEwan and Jackson 1996).

Hallock et al. (1961) reported that the composition of naturally-produced steelhead in the population estimates for the 1953-54 through 1958-59 seasons ranged from 82% to 97% and averaged 88%. This is probably not reflective of present composition in the Central Valley system, due to the large-scale loss of spawning and rearing habitat and increase in hatchery production. Today, four Central Valley steelhead hatcheries (Mokelumne River, Feather River, Coleman and Nimbus hatcheries) collectively produce approximately 1.5 million steelhead yearlings annually. There has been a substantial history of egg importation from non-native steelhead strains into these hatcheries (McEwan and Jackson 1996), but the degree of introgression with the native stock is largely unknown. Nimbus Hatchery and American River naturally spawning steelhead are thought to be highly introgressed with the native stock or perhaps have replaced the native population altogether (McEwan and Nelson 1991).

In response to the steelhead decline and decline of other Central Valley aquatic species, considerable planning efforts have begun recently to bring about recovery of steelhead, other anadromous fishes, and other elements of Central Valley aquatic ecosystems. The two most notable planning efforts are the Central Valley Project Improvement Act and the CALFED Bay-Delta program. Both of these projects have outlined recovery measures for steelhead on a basin-wide level. What is lacking in the outlined recovery measures and monitoring and research programs is a comprehensive baseline analysis of Central Valley steelhead genetics.

From 1994 through 1997, the National Marine Fisheries Service (NMFS) conducted genetic research using protein electrophoresis on a limited number of Central Valley steelhead populations, as part of a comprehensive coastwide status review of west coast steelhead to delineate population units that could be considered for listing under the Endangered Species Act (ESA). They analyzed samples of rainbow trout/steelhead collected from Coleman, Feather River, and Nimbus hatcheries, Mill and Deer creeks, and the American and Stanislaus rivers. Their analysis found, with the exception of the American river and Nimbus Hatchery, that these populations form a closely related group that is distinct from all other samples of steelhead that were analyzed (Busby et al. 1996; NMFS 1997).

The broad scope of the NMFS study (coastwide) provided information that was useful for purposes of delineation of Evolutionarily Significant Units (ESU's), but did not have the resolution necessary to provide meaningful information within ESU's, such as the Central Valley. There is a need for a more comprehensive evaluation that will augment the NMFS analysis and will provide essential information on the relationship of Central Valley steelhead populations to each other and to other populations of coastal rainbow trout.

The genetic and population information that will be generated from this evaluation will assist in determining those populations that may be native and the extent of introgression of non-native alleles from introduced hatchery stocks, and in estimating the structure and genetic variation within and among Central Valley steelhead populations. Since Central Valley steelhead were listed under the ESA, there have been several proposals from various water development and fisheries agencies to undertake genetic evaluations on specific streams of interest (e.g. the Stanislaus, Yuba, and Feather rivers and Clear Creek). Conducting a genetic evaluation on a stream-by-stream basis is a piecemeal approach, may be repetitious, and may yield conclusions that are misinterpreted because they are not interpreted or compared in the broader context of a basin-wide genetic profile. A comprehensive, basin-wide evaluation could provide the context for successful interpretation of genetic relationships of steelhead populations in these specific streams.

**Proposed Scope of Work:** The purpose of this project is to evaluate and describe the genetic and population structure and genetic variation of Central Valley steelhead populations by analyzing mitochondrial DNA (mtDNA) and/or microsatellite DNA. A comprehensive, basin-wide evaluation using analysis of DNA structure and allele frequencies will provide the context for successful interpretation of genetic relationships of steelhead populations in the Central Valley. Project timeline is from July 1999 to April 2002. A progress report will be done by September, 2000 and a final report and oral presentation of results will be done by April 30, 2002.

Collection of steelhead/rainbow trout tissue will be made from approximately 20 locations throughout the Sacramento/San Joaquin river system. Specific locations will include the American, Feather, Yuba, Stanislaus, Tuolumne, and upper Sacramento rivers and Dry (Placer County), Battle, Butte, Antelope, Clear, Stony, and Putah creeks. Tissue from 50 to 100 individuals will be collected from each site using standard methods of collection (electrofishing, hook and line, fyke net, rotary screw trap, beach seining) and genetic collection protocols to ensure that a representative sample from each population is obtained. Tissue (most likely from the caudal fin) will be taken and the fish returned unharmed to the stream of origin. Collecting tissue samples will be the responsibility of the Department of Fish and Game (Department) and will be made by, or directly supervised by, Department personnel. Microsatellite DNA and/or mtDNA will be analyzed at the U.S. Geological Survey, Biological Resources Division Lab in Anchorage Alaska by Dr. Jennifer Nielsen using standard techniques, and results will be compared to results from previously sampled populations that have already been analyzed (Mokelumne, Stanislaus, and American rivers; Mill and Deer creeks; and Coleman, Feather River and Nimbus hatcheries). Comparison with outgroups, such as southern California, north coast, and San Francisco Bay tributary steelhead populations and non-anadromous and domesticated hatchery rainbow trout, will also be done and a dendrogram will be constructed to show genetic relationships.

There will be three phases to the evaluation, described below.

#### Phase 1. Data Collection - July 1999 through August 2001

##### Task 1. Develop collection protocols and identify target populations

Specific populations of steelhead and resident rainbow trout will be identified in consultation with the Interagency Ecological Program Steelhead Project Work Team,

other agency biologists and other knowledgeable people. Criteria for choosing populations to be sampled will be developed and will include: steelhead populations of high interest (because of water operations or specific restoration projects); steelhead populations that could have metapopulation significance; non-anadromous rainbow trout populations that may be derived from remnant steelhead that are isolated above artificial barriers (but below natural barriers) that are self-sustaining and may exhibit adfluvial or potamodromous migratory behaviors.

#### Task 2. Tissue collection

It is anticipated that tissue will be collected mostly from June through November because high flows of winter and spring will likely make sampling difficult. Tissues will be collected and preserved according to established protocol, and a portion will be archived in the Department's Central Valley Salmonid Tissue Archive in Rancho Cordova.

#### Phase 2. Data Analysis - September 1999 through September 2001

This phase of the study will begin as soon as the first samples are collected and sent and will proceed concurrent with the data collection phase.

#### Phase 3. Report Preparation and Presentation of Results - September 2001 through April 2002

A progress report on data collection and any preliminary results from data analysis will be completed by September 30, 2000. A final report and oral presentation of results will be completed by April 30, 2002. The final report will provide conclusions regarding the four objectives of the evaluation, as described in the *Ecological/Biological Benefits* section of this proposal, will include a dendrogram or cladogram depicting phylogenetic relationships, and will include standard measures of genetic variation.

**Location and/or Geographic Boundaries of the Project:** The location of this evaluation is the entire Central Valley Bay-Delta watershed, as defined by the Ecosystem Restoration Plan (ERP) geographic scope (page 6 of ERPP, vol 1). However, to elucidate fully the phylogenetic relationships of Central Valley rainbow trout/steelhead, reaches that are presently inaccessible to steelhead because of artificial barriers will be sampled. These may include, but are not limited to, headwater reaches of the M.F. American, Feather, Yuba, and Stanislaus rivers and Battle, Clear, Stony, and Putah creeks.

#### **Ecological/Biological Benefits**

**Ecological/Biological Objectives:** A genetic evaluation of Central Valley steelhead populations would provide needed information on the phylogenetic relationships among putative native rainbow trout, naturally spawning steelhead, and hatchery steelhead that were founded from non-native broodstock. This information will be useful in estimating the structure and genetic variation within and among Central Valley steelhead populations.

Specific objectives of the evaluation are to:

- 1) Describe and compare genetic profiles and phylogenetic relationship of Central Valley naturally-spawning steelhead with other naturally spawning populations coastwide.
- 2) Describe and compare genetic profiles and phylogenetic relationship of steelhead populations of specific stream systems within the Central Valley.
- 3) Describe genetic profiles of Central Valley hatchery steelhead populations and compare to naturally-spawning, putative native populations of steelhead and isolated resident rainbow trout.
- 4) Analyze genotypes of self-sustaining, putative native Central Valley rainbow trout populations that are presently isolated above artificial barriers to determine their phylogenetic relationship to anadromous and stream-dwelling rainbow trout populations and strains.
- 5) Evaluate genetic variation of Central Valley naturally-spawning steelhead populations.

A primary benefit of this evaluation would be to identify the most appropriate steelhead stock to use as donors for reintroduction of steelhead to stream systems where they are thought to be extirpated. For example, there is a separate ongoing restoration effort to remove McCormick/Saeltzer Dam, which is preventing steelhead from accessing spawning and rearing habitat on Clear Creek. The steelhead population in Clear Creek is either very low or is nonexistent and reintroduction may become necessary if there is not a positive response in the steelhead population after the dam is removed. Therefore, if reintroduction becomes necessary, then the most genetically appropriate stock must be identified. Potential donor stocks may include putative native rainbow trout populations that were recently isolated from the ocean due to construction of impassable dams. Identification of these isolated populations, if they exist, could greatly enhance our ability to reintroduce native steelhead to Central Valley streams where they have become extirpated.

Another primary benefit would be to elucidate the phylogenetic relationship of natural stocks with hatchery stocks. This would allow us to 1) assess whether hatchery practices are having unintended genetic effects on the natural populations, such as introgression and resultant outbreeding depression, and 2) assess whether hatchery populations are significantly different from the natural populations from which they were founded.

A secondary benefit would be the enhancement of knowledge of the relationship of individual Central Valley steelhead populations to each other, and the relationship to the non-migratory rainbow trout forms that are sympatric with the steelhead forms or exist in headwater reaches above impassable dams.

Another secondary benefit would be to obtain measures of the genetic variation (proportion of polymorphic loci, average expected heterozygosity, average number of alleles per locus) *within* Central Valley steelhead populations, as well as divergence *among* populations. This would allow



resource managers to determine if there has been a reduction of genetic diversity in Central Valley steelhead populations.

**Linkages:** Other agencies that have expressed interest in obtaining genetic information on steelhead on specific streams include the Department of Water Resources (Feather River), Yuba County Water Agency (Yuba River), the U.S. Fish and Wildlife Service (Clear Creek, upper Sacramento River), CALFED ERP (Battle Creek), NMFS (basin-wide) and local water districts on the Stanislaus River. Information obtained from this genetic analysis will be available to these parties.

This project, as proposed, will be cost-shared using Central Valley Project Improvement Act (CVPIA) Anadromous Fish Restoration Program (AFRP) funds (43%), CALFED Category 3 funds (41%), and Department funds (16%). AFRP funding for this project has been approved in concept by the AFRP Restoration Coordinators and is in the current FY federal budget. The Department cost share is in personnel cost (salary, benefits, and overhead) of the principal investigator for planning and tissue collection.

A *Strategic Objective* for steelhead, as stated in volume 1 of the ERPP (pg. 229, Feb 1999 version) is to "determine the abundance, distribution, and structure of existing steelhead populations" (emphasis added). A stated action (page 230) is to "manage and operate the four hatcheries in the Central Valley that propagate steelhead in order to protect the genetic diversity of naturally and hatchery produced stocks..." A comprehensive genetic evaluation is necessary to determine population structure of Central Valley steelhead and to provide the baseline information to measure genetic impacts to natural stocks from hatchery operations and practices. Information from this evaluation will be useful in the upcoming NMFS evaluation of hatchery practices.

The *Comprehensive Monitoring, Assessment, and Research Program* (CMARP) Draft Plan (page 40) identifies six major knowledge gaps for Central Valley steelhead "requiring either new monitoring and assessment programs or enhancements to ongoing anadromous fish monitoring programs." "Genetic and population structure" is one of the identified knowledge gaps. Technical Appendix VII.A-11 (Monitoring, Assessment, and Research on Central Valley Steelhead: Status of Knowledge, Review of Existing Programs, and Assessment of Needs - page 26) of the CMARP plan states:

"A genetic evaluation of Central Valley steelhead populations is necessary to determine phylogenetic relationships among putative native rainbow trout, naturally spawning steelhead, and hatchery steelhead that were founded from non-native broodstock. This information will be useful in estimating the structure and genetic diversity within and among Central Valley steelhead populations. A comprehensive, basin-wide evaluation using analysis of mtDNA and microsatellite DNA structure and allele frequencies could provide the context for successful interpretation of genetic relationships of steelhead populations in these specific streams."

Steelhead are a high priority listed species, as stated in the CALFED 1999 Action Plan (Action Plan). *Improved Fish Management and Hatchery Operations* is a Topic Area for which the PSP is soliciting proposals. The Action Plan states: "Proposed actions to restore anadromous fish to

*former habitat areas need to be guided by genetic and ecological analyses. Objectives of these analyses would include ensuring the genetic diversity and demographic viability of founder populations”* This proposal will provide the basis for genetic analyses and ensuring that appropriate stocks are used as founder populations.

A Focused Action of the Action Plan is to: *“Develop biological and genetic management plans to address restoration and recolonization of streams in the Central Valley by chinook salmon and steelhead.”* A comprehensive genetic evaluation for Central Valley steelhead will provide the basis for, and will be a substantial part of, genetic management plans that will be developed for each watershed or stream restoration project. Elements, as stated in the Action Plan (pg 28), that will be addressed by this proposal include:

- ▶ stock- and species-specific guidelines for genetic conservation and management
- ▶ long-term genetic monitoring plan
- ▶ identification of appropriate source populations to....accelerate recolonization.

#### **Technical Feasibility and Timing**

An alternative to this approach is to continue to augment the current data set using the same methodology (protein electrophoresis) as the previously mentioned NMFS genetic analysis. However, this methodology requires a large amount of tissue and requires the sacrifice of fish specimens, hence can be detrimental to small populations. Because some Central Valley steelhead populations are quite small and all are listed under the ESA, continuing to utilize this method is not feasible.

If and when ESA Section 9 take prohibitions are applied to Central Valley steelhead, it is anticipated that collection of specimens will be permitted through a forthcoming ESA 4(d) Rule that will authorize all Department steelhead monitoring and research activities, hence no additional incidental take permits or environmental documents will be required for this activity. If this is not the case, however, then we will pursue obtaining a Section 10 incidental take permit. Sample collection will be coordinated with the U.S Fish and Wildlife Service (Service) to ensure that methods used to obtain specimens will not harm other listed species, and will obtain a Section 10 incidental take permit if necessary (this may be covered under the Department’s Section 6 agreement with the Service).

#### **Monitoring and Data Collection Methodology**

**Biological/Ecological Objectives:** The primary questions to be addressed by this evaluation, as they relate to the stated objectives are shown in Table 1.

**Monitoring Parameters and Data Collection Approach:** The data collection approach to address the above questions will be as follows: Tissue will be collected from approximately 20 populations throughout the Central Valley watershed. Collections will be made from populations in stream reaches that are accessible to steelhead, hatchery populations, and rainbow trout that are presently isolated above artificial barriers. Collection will focus mostly on juvenile fish because they are more readily available, however, tissue from adult steelhead will be collected whenever possible.

An attempt will be made to represent all juvenile year-classes present at a location. Fish will be captured by standard methods (electrofishing, hook and line, fyke net, rotary screw trap, beach seining) from several locations in the sampled reach to minimize collection of sibling groups. Tissue from approximately 50 to 100 individuals will be collected (most likely from the caudal fin), fixed, and transmitted to the genetics lab. Fish will be returned to the stream after the tissue is obtained.

**Data Evaluation Approach.** DNA will be extracted, amplified, and analyzed using standard methods. Comparisons will be made with other populations of coastal rainbow trout (*O.m. irideus*), other populations of rainbow trout (*O. m. spp.*), and among the Central Valley samples to determine if there are differences within and among the populations. Allelic differences will be compared, and a dendrogram or cladogram will be constructed to show genetic/taxonomic relationships. Measures of genetic variation (proportion of polymorphic loci, average expected heterozygosity, average number of alleles per locus) *within* Central Valley steelhead will be analyzed to determine if genetic diversity has been reduced.

*Analysis of molecular variance (AMOVA analysis from ARLEQUIN)* based on standard  $F_{st}$  values will be used to compare genetic diversity among and between steelhead populations at several geographic scales, i.e. regional within California, basin specific, stream population, and individual fish samples. A surrogate measure of gene flow ( $N_m$ ; Slatkin 1995) between all possible pairs of steelhead will be calculated based on the number of alleles by ARLEQUIN (Schneider et al. 1997). Pairwise multilocus  $F_{st}$  estimates will be correlated to waterway distances in a test of isolation-by-distance, and the inverse regression slope of multilocus  $F_{st}$  values against stream-distance will be used to estimate dispersal distance over one generation (Rousset 1997). Pairwise genetic distance matrices will be calculated using delta mu squared and Nei's standard genetic distance (MICROSAT; Minch 1998). Genetic distance data will be used to generate unrooted consensus neighbor-joining trees using NEIGHBOR and CONSENSE applications from PHYLIP (Felsenstein 1993). Ten thousand replicate genetic distance trees will be calculated to obtain branching bootstrap values for the consensus trees.

If statistical analysis fails to detect differentiation among one or more individual populations, an analysis of statistical power (Dizon 1995) may be done to determine if this is a function of there being none or if it's a function of experimental design.

A progress report on data collection and preliminary results from data analysis will be done by September 30, 2000. A final report and oral presentation of results will be done by April 30, 2002. The final report will provide conclusions regarding the objectives of the evaluation, as described in the *Expected Benefits* section of this proposal, will include a dendrogram or cladogram depicting phylogenetic relationships, and will include standard measures of genetic variation

Table 1. Monitoring and Data Collection Information

| Hypothesis/question to be Evaluated                                                                                                                                                  | Monitoring Parameter(s) and Data Collection Approach                                                                                                                                                                                                                                                                                                                                                                                                                          | Data Evaluation Approach                                                                                                                                                                                                                                                                                                                                                                                                                                                | Comments/Data Priority                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Objective 1: Describe and compare genetic profile and phylogenetic relationship of Central Valley naturally spawning steelhead with naturally spawning populations coastwide.</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Are Central Valley steelhead genetically distinct from other coastal steelhead populations?                                                                                          | Tissue will be collected from app. 20 populations throughout the Central Valley watershed. Collection will focus mostly on juvenile fish with an attempt to collect from all year-classes present. Fish will be captured by standard methods from several locations in the sampled reach to avoid collecting sibling groups. Tissue from approximately 50 to 100 individuals will be collected (most likely from the caudal fin), fixed, and transmitted to the genetics lab. | Microsatellite and/or mtDNA will be extracted, amplified, and analyzed using standard methods. Comparisons will be made to other populations of coastal rainbow trout ( <i>O.m. irideus</i> ) and other populations of rainbow trout ( <i>O. m. spp</i> ) that have been analyzed (including inland rainbow trout populations and hatchery strains). Allelic differences will be compared, and a cladogram will be constructed to show genetic/taxonomic relationships. | The NMFS protein electrophoretic analysis found that several populations of Central Valley steelhead show close genetic affinities to each other and they form a genetic group that is distinct from all other samples of steelhead analyzed (Busby et al. 1996; NMFS 1997). Because DNA analysis provides greater resolution than protein electrophoresis, it is expected that differences will be discernable through microsatellite and/or mtDNA techniques. |
| <b>Objective 2: Describe and compare genetic profiles and phylogenetic relationship of steelhead populations of specific stream systems within the Central Valley.</b>               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Is variation <i>within</i> Central Valley steelhead populations discernable at the molecular genetic level?                                                                          | Same as Objective 1.                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Same as Objective 1. Allelic variation of all Central Valley sampled populations will be compared with each other to determine if there is genetic variation within the Central Valley steelhead metapopulation.                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

| Hypothesis/question to be Evaluated                                                                                                                                                                                                                                                           | Monitoring Parameter(s) and Data Collection Approach                                                                                                                                                                                                                                                                                    | Data Evaluation Approach                                                                                                                                                                                                                                          | Comments/Data Priority                                                                                                                                                                                                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Objective 3: Describe genetic profiles of Central Valley hatchery steelhead populations and compare to naturally-spawning, putative native populations of steelhead and isolated resident rainbow trout.</b>                                                                               |                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                        |
| Are hatchery steelhead populations genetically distinct from naturally-spawning or putative native populations?                                                                                                                                                                               | Same as Objective 1. The four Central Valley hatchery populations will be included in the sampled populations                                                                                                                                                                                                                           | Same as Objective 1. Allelic variation of all Central Valley natural populations sampled will be compared with the hatchery populations to determine if there are genetic differences                                                                             | The NMFS protein electrophoretic study provides some evidence that Nimbus Hatchery steelhead are distinct from putative native Central Valley steelhead, whereas Coleman and Feather River Hatchery steelhead are not. |
| <b>Objective 4: Analyze genotypes of self-sustaining, putative native Central Valley rainbow trout populations that are presently isolated above artificial barriers to determine their phylogenetic relationship to anadromous and stream-dwelling rainbow trout populations and strains</b> |                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                        |
| Are non-anadromous rainbow trout populations presently isolated from anadromous forms by artificial barriers genetically distinct from steelhead populations below the barriers?                                                                                                              | Same as Objective 1. Tissue will be collected from non-anadromous rainbow trout populations in the M.F American River, the Yuba River above Englebright Reservoir, Clear Creek above Whiskeytown Reservoir, upper Stony Creek, Putah Creek above Lake Berryessa, the Stanislaus River above New Melones Reservoir, and other locations. | Same as Objective 1. Allelic variation of all sampled Central Valley steelhead will be compared with the rainbow trout populations above the barriers to determine if there are genetic differences                                                               | Molecular genetic analysis of isolated populations of rainbow trout in Southern California has shown that these populations are similar to steelhead populations that exist below the barrier.                         |
| <b>Objective 5: Evaluate genetic variation of Central Valley naturally-spawning steelhead populations.</b>                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                        |
| What is the level of genetic variability within Central Valley steelhead populations?                                                                                                                                                                                                         | Same as Objective 1.                                                                                                                                                                                                                                                                                                                    | Same as Objective 1. Measures of the genetic variability (proportion of polymorphic loci, average expected heterozygosity, average number of alleles per locus) within Central Valley steelhead will be analyzed to determine if genetic diversity has been lost. |                                                                                                                                                                                                                        |

### **Local Involvement**

Organizations/agencies that are aware of this proposal and have shown interest include: the Department of Water Resources; the U.S. Fish and Wildlife service; and Jones and Stokes, Associates. No organizations have expressed opposition to this project, and, because it involves only data collection and analysis, it is anticipated that there will be no opposition to the proposal. No third party impacts are anticipated.

### **Cost**

**Budget:** CALFED funds are needed primarily to provide temporary help (16 personnel-months) and travel costs for Department scientific aids to assist with tissue collection, minor equipment, and a portion of the Service Contract with the genetics lab for data analysis (see *Cost Sharing*, below). Benefits for scientific aids are calculated at 7.66% and overhead is calculated at 17.2%.

The budget for the entire project is shown in Table 2. The budget for CALFED requested funds is shown in Table 3.

### **Cost Sharing**

This project, as proposed, will be cost-shared using Central Valley Project Improvement Act (CVPIA) Anadromous Fish Restoration Program (AFRP) funds (43%), CALFED Category 3 funds (41%), and Department funds (16%). A total of \$75,000 from AFRP funding has been requested and has been approved in concept by the AFRP Restoration Coordinators. It is in the current FY federal budget. The Department cost share is in personnel cost (salary, benefits, and overhead) of the principal investigator for planning and tissue collection.

Table 2. Total Budget for Project

| Project Phase and Task                                 | Direct Labor Hours                                        | Direct Salary and Benefits | Service Contracts | Material and Acquisition Costs | Misc. and other Direct Costs  | Overhead and Indirect Costs | Total Cost            |
|--------------------------------------------------------|-----------------------------------------------------------|----------------------------|-------------------|--------------------------------|-------------------------------|-----------------------------|-----------------------|
| <b>Data Collection</b>                                 |                                                           |                            |                   |                                |                               |                             |                       |
| Task 1 - Develop protocols and i.d. target populations | <sup>1</sup> (1) Senior Biologist: (0.5 pm <sup>2</sup> ) | \$ 3,245                   |                   |                                |                               | \$558                       | <sup>1</sup> \$ 3,803 |
| Task 2 - Tissue Collection                             | <sup>1</sup> (1) Senior Biologist: (3 pm <sup>2</sup> )   | \$19,470                   |                   |                                | travel (@ \$50/day): \$ 2,000 | \$3,349                     | <sup>1</sup> \$24,819 |
|                                                        | (4) Sci. Aids: (16 pm <sup>2</sup> )                      | \$24,860                   |                   |                                | travel (@ \$50/day): \$8,000  | \$4,276                     | \$37,136              |
|                                                        |                                                           |                            |                   |                                | misc. minor equip: \$ 500     |                             | \$ 500                |
| <b>Data Analysis and Report Writing</b>                |                                                           |                            | \$108,000         |                                |                               |                             | \$ 108,000            |
| <b>TOTAL COST OF PROJECT:</b>                          |                                                           |                            |                   |                                |                               |                             | \$174,258             |
| <b>DFG COST SHARE:</b>                                 |                                                           |                            |                   |                                |                               |                             | \$28,622              |
| <b>CALFED FUNDS REQUESTED:</b>                         |                                                           |                            |                   |                                |                               |                             | \$70,636              |
| <b>AFRP FUNDS REQUESTED:</b>                           |                                                           |                            |                   |                                |                               |                             | \$75,000              |

<sup>1</sup> Cost for these positions will be provided by the Department as a cost-share for the project.<sup>2</sup> Personnel-month

Table 3. Total Budget for CALFED requested funds only.

| Project Phase and Task           | Direct Labor Hours                   | Direct Salary and Benefits | Service Contracts | Material and Acquisition Costs | Misc. and other Direct Costs                                 | Overhead and Indirect Costs | Total Cost      |
|----------------------------------|--------------------------------------|----------------------------|-------------------|--------------------------------|--------------------------------------------------------------|-----------------------------|-----------------|
| Task 2 - Tissue Collection       | (4) Sci. Aids: (16 pm <sup>1</sup> ) | \$24,860                   |                   |                                | travel (@ \$50/day): \$8,000<br><br>misc. minor equip: \$500 | \$4,276                     | \$37,636        |
| Data Analysis and Report Writing |                                      |                            | \$33,000          |                                |                                                              |                             | \$ 33,000       |
| <b>CALFED FUNDS REQUESTED:</b>   |                                      |                            |                   |                                |                                                              |                             | <b>\$70,636</b> |

<sup>1</sup> Personnel-month

Table 4. Quarterly Budget

| Task                           | Quarterly Budget Oct-Dec 99 | Quarterly Budget Jan-Mar 00 | Quarterly Budget Apr-Jun 00 | Quarterly Budget Jul-Sep 00 | Quarterly Budget Oct-Dec 00 |
|--------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Task 2 Tissue Collection       | \$4,705                     | \$4,705                     | \$4,705                     | \$4,705                     | \$4,705                     |
| Data Analysis and Report Prep. | \$3,000                     | \$3,000                     | \$3,000                     | \$3,000                     | \$3,000                     |
| <b>TOTAL</b>                   | <b>\$7,705</b>              | <b>\$7,705</b>              | <b>\$7,705</b>              | <b>\$7,705</b>              | <b>\$7,705</b>              |

Table 4. Quarterly Budget, cont.

| Task                           | Quarterly Budget Jan-Mar 01 | Quarterly Budget Apr-Jun 01 | Quarterly Budget Jul-Sep 01 | Quarterly Budget Oct-Dec 01 | Quarterly Budget Jan-Mar 02 | Quarterly Budget Apr-Jun 02 | <b>TOTAL BUDGET</b> |
|--------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------|
| Task 2 Tissue Collection       | \$4,705                     | \$4,705                     | \$4,705                     |                             |                             |                             | \$37,636            |
| Data Analysis and Report Prep. | \$3,000                     | \$3,000                     | \$3,000                     | \$3,000                     | \$3,000                     | \$3,000                     | \$33,000            |
| <b>TOTAL</b>                   | <b>\$7,705</b>              | <b>\$7,705</b>              | <b>\$7,705</b>              | <b>\$3,000</b>              | <b>\$3,000</b>              | <b>\$3,000</b>              | <b>\$70,636</b>     |



**Schedule:** See Table 5 for start/completion dates of specific tasks.

Table 5. Schedule milestones

|                                                   | 1999 |   |   |   |   | 2000 |   |   |   |   |   |   |   |   |   | 2001 |   |   |   |   |   |   |   |   |   | 2002 |   |   |   |   |   |   |   |   |   |   |   |
|---------------------------------------------------|------|---|---|---|---|------|---|---|---|---|---|---|---|---|---|------|---|---|---|---|---|---|---|---|---|------|---|---|---|---|---|---|---|---|---|---|---|
| Phase/Task                                        | j    | a | s | o | n | d    | j | f | m | a | m | j | j | a | s | o    | n | d | e | j | f | m | a | m | j | j    | a | s | o | n | d | e | j | f | m | a |   |
|                                                   | u    | u | e | c | e | a    | e | a | p | a | u | u | a | e | c | o    | v | e | a | e | p | a | u | u | a | e    | c | o | v | e | a | e | p | a | u | u | a |
| 1.1 Develop protocols and i.d. target populations |      |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |   |   |
| 1.2 Tissue Collection                             |      |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |   |   |
| 2.0 Data Analysis                                 |      |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |   |   |
| 3.0 Report Preparation                            |      |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   |   |   |   |

### Applicant Qualifications

#### Principle Investigator:

Dennis McEwan  
 Senior Fishery Biologist - Steelhead Trout Specialist  
 California Dept. of Fish and Game  
 1416 Ninth St.  
 Sacramento, CA. 95814  
 office: (916) 653-9442  
 fax: (916) 654-8099  
 email: dmcewan@hq.dfg.ca.gov

As the Project Leader for the Department's Steelhead Restoration and Management Project (from 3/91 to present), McEwan is responsible for coordinating and implementing restoration and management activities for steelhead and acting as the Department's steelhead expert on technical and management issues. McEwan was responsible for making several collections for the NMFS coastwide steelhead genetic analysis that was done as part of the Coastwide Steelhead Status Review. McEwan obtained a Msc. and Bsc. in Biology (emphasis on Biological Conservation) from California State University, Sacramento.

McEwan will be responsible for developing collection protocols, identification of target populations (in consultation with Nielsen), making or overseeing all sample collections, and

transmitting samples to the genetics lab. Collections will be coordinated with other agencies (Fish and Wildlife Service, DWR, Jones and Stokes, etc.) so that their information needs are met. Scientific Aids will be used to assist with sample collection.

**Geneticist:**

Dr. Jennifer Nielsen  
Fisheries Programs Supervisor  
U.S. Geological Survey  
Biological Resources Division  
1011 E. Tudor Rd.  
Anchorage, AK. 99503  
office: (907) 786-3670  
fax: (907) 786-3636  
email: jennifer\_nielsen@USGS.gov

Nielsen founded and supervised the J.L. Nielsen Fish Molecular and Conservation Genetics Laboratory at the Hopkins Marine Station, Stanford University. The lab primarily conducted research on evolutionary genetics in freshwater and anadromous fish populations of central and southern California and relic populations throughout the world. Nielsen is a recognized expert in Pacific salmonid molecular genetics and stock identification, and has done a considerable amount of work elucidating the genetic and population structure of coastal rainbow trout and steelhead by analyzing mtDNA and microsatellites. Nielsen obtained a Ph.D. from the University of California, Berkeley, in Environmental Science, Policy and Management, a MSc. from the University of California, Berkeley, in Wildlands Resource Sciences, and a BSc. from the Evergreen State College, WA. Nielsen's Ph.D dissertation was on Molecular Genetics and Stock Identification in Pacific Salmon (*Oncorhynchus* spp.)

Nielsen's previous genetic research includes: research on rainbow trout populations from southwest Alaska using DNA microsatellite probes; comparison of genetic approach drawn from allozymes and DNA for definition of population structure in southern steelhead; molecular genetic structure of steelhead and rainbow trout in the Santa Ynez River; molecular analyses of Mexican trout from the Rio Yaqui drainage; molecular genetics of the McCloud River redband trout; differences in genetic diversity for mtDNA between hatchery and wild populations of *Oncorhynchus*; phylogeographic patterns in California steelhead using mtDNA and microsatellites; biogeographic distributions of mitochondrial and nuclear markers for southern steelhead; upper Sacramento River Rainbow trout: molecular genetics and stock identification. Nielsen will be responsible for all aspects of the genetic analysis and report preparation.

**Literature Cited**

Busby, P.J., T.C. Wainwright, G.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz, and I. V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-NWFSC-27.

California Department of Fish and Game. 1965. California Fish and Wildlife Plan.

Dizon, A. E., Taylor, B. L., and O'Corry-Crowe, G. M. Why statistical power is necessary to link analyses of molecular variation to decisions about population structure. Pages 288 -294 in J. L. Nielsen, editor. Evolution and the Aquatic Ecosystem: defining unique units in population conservation. American Fisheries Society Symposium 17, Bethesda, Maryland)

Hallock, R.J., W.F. Van Woert, and L. Shapovalov. 1961. An evaluation of stocking hatchery-reared steelhead rainbow trout (*Salmo gairdnerii gairdnerii*) in the Sacramento River system. Calif. Dept. Fish and Game Fish Bull. No. 114. 74 pp.

McEwan, D. and T.A. Jackson. 1996. Steelhead Restoration and Management Plan for California. Calif. Dept. of Fish and Game.

National Marine Fisheries Service. 1997. Status review update for deferred and candidate ESUs of west coast steelhead. Prepared by NMFS West Coast Steelhead Biological Review Team.

Yoshiyama, R.M., E.R. Gerstung, F.W. Fisher, and P.B. Moyle. 1996. Historical and present distribution of chinook salmon in the Central Valley drainage of California. Sierra Nevada Ecosystem Project: Final report to Congress, vol.III. Centers for Water and Wildland Resources, Univ. Cal. Davis. pg. 309-361.



State of California - The Resources Agency

**DEPARTMENT OF FISH AND GAME**

<http://www.dfg.ca.gov>

1416 Ninth Street  
Sacramento, CA 95814

PETE WILSON, Governor



(916) 653-9442

email: [dmcewan@hq.dfg.ca.gov](mailto:dmcewan@hq.dfg.ca.gov)

April 12, 1999

Sacramento County Board of Supervisors  
700 H St.  
Sacramento, CA. 95814

To Whom It May Concern:

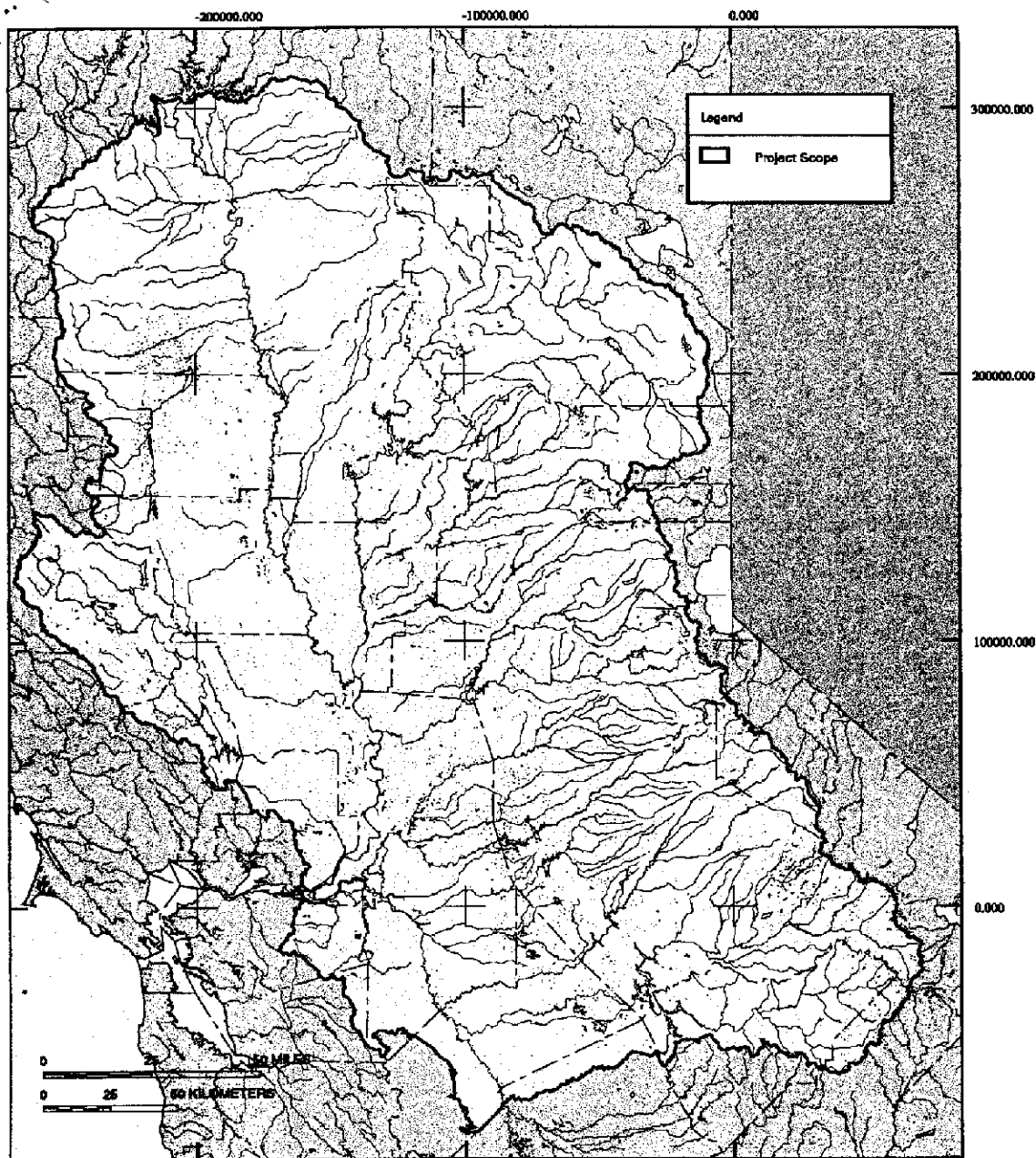
I am writing to notify the County of Sacramento that I, Dennis McEwan, am submitting a grant application for CALFED Ecosystem Restoration funding to conduct a comprehensive genetic evaluation of Central Valley steelhead trout. A requirement of the grant application is that the applicant provide notification to the local County Board of Supervisors and Planning Department of their intent to submit an application. If you have any questions, please contact me.

Sincerely,

Dennis McEwan  
Senior Fishery Biologist  
Watershed Restoration Branch

cc: Sacramento County Planning Department  
Delta Protection Commission  
Bay Conservation and Development Commission

*Conserving California's Wildlife Since 1870*



# Geographic Scope of Central Valley Steelhead Genetic Evaluation

1:2000000

April 14, 1998

Fisheries Programs Branch

Transverse Mercator  
Projection: Albers  
Units: Meters  
Datum: NAD27  
1st standard parallel: 34 0 0.000  
2nd standard parallel: 40 30 0.000  
Central meridian: -120 0 0.000  
Latitude of projection's origin: 0 0 0.000  
False easting (meters): 0.000000  
False northing (meters): -4000000.000000

**APPLICATION FOR  
FEDERAL ASSISTANCE**

OMB Approval No. 0348-0043

|                                                                                                                                                                                                                                                       |  |                                     |                      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-------------------------------------|----------------------|
| <b>1. TYPE OF SUBMISSION:</b><br>Application<br><input type="checkbox"/> Construction<br><input checked="" type="checkbox"/> Non-Construction<br>Preapplication<br><input type="checkbox"/> Construction<br><input type="checkbox"/> Non-Construction |  | <b>2. DATE SUBMITTED</b><br>4/16/99 | Applicant Identifier |
| <b>3. DATE RECEIVED BY STATE</b>                                                                                                                                                                                                                      |  | State Application Identifier        |                      |
| <b>4. DATE RECEIVED BY FEDERAL AGENCY</b>                                                                                                                                                                                                             |  | Federal Identifier                  |                      |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|--------|----|--------------|----|--------|----|----------|----|--|----|----------|----|--|----|----------|----|--------|----|-------------------|----|--|----|----------|----|---------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>5. APPLICANT INFORMATION</b><br>Legal Name: <u>DENNIS R. McEWAN</u><br>Address (give city, county, State, and zip code):<br><u>1416 Ninth St.</u><br><u>Sacramento, CA 95814</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Organizational Unit: <u>CALIFORNIA DEPT. OF FISH &amp; GAME</u><br><u>WATERSHED RESTORATION BRANCH</u><br>Name and telephone number of person to be contacted on matters involving this application (give area code):<br><u>DENNIS McEWAN</u><br><u>916 653-9442</u> |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>6. EMPLOYER IDENTIFICATION NUMBER (EIN):</b><br><div style="border: 1px solid black; padding: 2px; display: inline-block;">             94-1697567           </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | <b>7. TYPE OF APPLICANT: (enter appropriate letter in box)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 45%;">             A. State<br/>             B. County<br/>             C. Municipal<br/>             D. Township<br/>             E. Interstate<br/>             F. Intermunicipal<br/>             G. Special District           </div> <div style="width: 45%;">             H. Independent School Dist.<br/>             I. State Controlled Institution of Higher Learning<br/>             J. Private University<br/>             K. Indian Tribe<br/>             L. Individual<br/>             M. Profit Organization<br/>             N. Other (Specify) _____           </div> </div> <div style="text-align: right; margin-top: -20px;"> <div style="border: 1px solid black; padding: 2px; display: inline-block;">A</div> </div> |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>8. TYPE OF APPLICATION:</b><br><div style="display: flex; justify-content: space-around;"> <input checked="" type="checkbox"/> New           <input type="checkbox"/> Continuation           <input type="checkbox"/> Revision         </div> If Revision, enter appropriate letter(s) in box(es) <div style="display: inline-block; width: 20px; height: 20px; border: 1px solid black; margin: 0 5px;"></div> <div style="display: inline-block; width: 20px; height: 20px; border: 1px solid black; margin: 0 5px;"></div><br>A. Increase Award    B. Decrease Award    C. Increase Duration<br>D. Decrease Duration    Other(specify): _____                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:</b><br><div style="display: flex; align-items: center;"> <div style="border: 1px solid black; width: 20px; height: 20px; margin-right: 5px;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; margin-right: 5px;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; margin-right: 5px;"></div> <div style="border: 1px solid black; width: 20px; height: 20px; margin-right: 5px;"></div> </div> TITLE: _____                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:</b><br><u>CENTRAL VALLEY STEELHEAD</u><br><u>GENETIC EVALUATION</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>12. AREAS AFFECTED BY PROJECT (Cities, Counties, States, etc.):</b><br><u>CENTRAL VALLEY (SACRAMENTO &amp; SAN JOAQUIN RIVER WATERSHEDS)</u><br><u>- CALIFORNIA</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>13. PROPOSED PROJECT</b><br>Start Date: <u>7/99</u> Ending Date: <u>4/02</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <b>14. CONGRESSIONAL DISTRICTS OF:</b><br>a. Applicant: <u>5</u><br>b. Project: <u>STATEWIDE</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>15. ESTIMATED FUNDING:</b><br><table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 20%;">a. Federal</td> <td style="width: 10%;">\$</td> <td style="width: 40%; text-align: center;">70,636</td> <td style="width: 10%; text-align: right;">00</td> </tr> <tr> <td>b. Applicant</td> <td>\$</td> <td style="text-align: center;">28,622</td> <td style="text-align: right;">00</td> </tr> <tr> <td>c. State</td> <td>\$</td> <td></td> <td style="text-align: right;">00</td> </tr> <tr> <td>d. Local</td> <td>\$</td> <td></td> <td style="text-align: right;">00</td> </tr> <tr> <td>e. Other</td> <td>\$</td> <td style="text-align: center;">75,000</td> <td style="text-align: right;">00</td> </tr> <tr> <td>f. Program Income</td> <td>\$</td> <td></td> <td style="text-align: right;">00</td> </tr> <tr> <td>g. TOTAL</td> <td>\$</td> <td style="text-align: center;">174,258</td> <td style="text-align: right;">00</td> </tr> </table> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | a. Federal                                                                                                                                                                                                                                                           | \$                                                              | 70,636 | 00 | b. Applicant | \$ | 28,622 | 00 | c. State | \$ |  | 00 | d. Local | \$ |  | 00 | e. Other | \$ | 75,000 | 00 | f. Program Income | \$ |  | 00 | g. TOTAL | \$ | 174,258 | 00 | <b>16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?</b><br>a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:<br>DATE _____<br>b. No. <input type="checkbox"/> PROGRAM IS NOT COVERED BY E. O. 12372<br><input type="checkbox"/> OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW |
| a. Federal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 70,636                                                                                                                                                                                                                                                               | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| b. Applicant                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 28,622                                                                                                                                                                                                                                                               | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| c. State                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                      | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| d. Local                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                      | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| e. Other                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 75,000                                                                                                                                                                                                                                                               | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| f. Program Income                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                      | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| g. TOTAL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | \$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 174,258                                                                                                                                                                                                                                                              | 00                                                              |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?</b><br><input type="checkbox"/> Yes If "Yes," attach an explanation. <input checked="" type="checkbox"/> No                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT, THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                      |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| a. Type Name of Authorized Representative<br><u>Dennis McEwan</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | b. Title<br><u>Alt. Baracua Senior Budget Specialist</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | c. Telephone Number<br><u>916 653-9442</u>                                                                                                                                                                                                                           | d. Signature of Authorized Representative<br><u>[Signature]</u> |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |
| e. Date Signed<br><u>4/15/99</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Standard Form 424 (Rev. 7-97)<br>Prescribed by OMB Circular A-102                                                                                                                                                                                                    |                                                                 |        |    |              |    |        |    |          |    |  |    |          |    |  |    |          |    |        |    |                   |    |  |    |          |    |         |    |                                                                                                                                                                                                                                                                                                                                                                                     |

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## BUDGET INFORMATION - Non-Construction Programs

## SECTION A - BUDGET SUMMARY

| Grant Program Function or Activity (a) | Catalog of Federal Domestic Assistance Number (b) | Estimated Unobligated Funds |                 | New or Revised Budget |                 |            |
|----------------------------------------|---------------------------------------------------|-----------------------------|-----------------|-----------------------|-----------------|------------|
|                                        |                                                   | Federal (c)                 | Non-Federal (d) | Federal (e)           | Non-Federal (f) | Total (g)  |
| 1.                                     |                                                   | \$                          | \$              | \$ 70,636             | \$ 103,622      | \$ 174,258 |
| 2.                                     |                                                   |                             |                 |                       |                 |            |
| 3.                                     |                                                   |                             |                 |                       |                 |            |
| 4.                                     |                                                   |                             |                 |                       |                 |            |
| 5. Totals                              |                                                   | \$                          | \$              | \$ 70,636             | \$ 103,622      | \$ 174,258 |

## SECTION B - BUDGET CATEGORIES

| 6. Object Class Categories             | GRANT PROGRAM, FUNCTION OR ACTIVITY |     |     |     | Total (5)  |
|----------------------------------------|-------------------------------------|-----|-----|-----|------------|
|                                        | (1)                                 | (2) | (3) | (4) |            |
| a. Personnel                           | \$ 40,287                           | \$  | \$  | \$  | \$         |
| b. Fringe Benefits                     | 7,288                               |     |     |     |            |
| c. Travel                              | 10,000                              |     |     |     |            |
| d. Equipment                           |                                     |     |     |     |            |
| e. Supplies                            | 500                                 |     |     |     |            |
| f. Contractual                         | 108,000                             |     |     |     |            |
| g. Construction                        |                                     |     |     |     |            |
| h. Other                               |                                     |     |     |     |            |
| i. Total Direct Charges (sum of 6a-6h) | 166,075                             |     |     |     |            |
| j. Indirect Charges                    | 8,183                               |     |     |     |            |
| k. TOTALS (sum of 6i and 6j)           | \$ 174,258                          | \$  | \$  | \$  | \$ 174,258 |
| 7. Program Income                      | \$                                  | \$  | \$  | \$  | \$         |

Applicant (state) contribution is in-kind personnel cost, overhead, and travel for the Principal Investigator.



| SECTION 1 - BUDGET ESTIMATES OF NEED FOR FUNDING NEEDS FOR THE PROJECT |                       |             |                   |             |             |
|------------------------------------------------------------------------|-----------------------|-------------|-------------------|-------------|-------------|
| (a) Grant Program                                                      | (b) Applicant         | (c) State   | (d) Other Sources | (e) TOTALS  |             |
| 8.                                                                     | \$ 28622              | \$          | \$                | \$          |             |
| 9.                                                                     |                       |             | 75,000            |             |             |
| 10.                                                                    |                       |             |                   |             |             |
| 11.                                                                    |                       |             |                   |             |             |
| 12. TOTAL (sum of lines 8 - 11)                                        | \$ 28622              | \$          | \$ 75,000         | \$ 103,622  |             |
| SECTION 2 - BUDGET ESTIMATES OF NEED FOR FUNDING NEEDS FOR THE PROJECT |                       |             |                   |             |             |
|                                                                        | Total for 1st Year    | 1st Quarter | 2nd Quarter       | 3rd Quarter | 4th Quarter |
| 13. Federal                                                            | \$                    | \$ 3159     | \$ 3159           | \$ 3159     | \$ 3159     |
| 14. NonFederal                                                         |                       | 4546        | 4546              | 4546        | 4546        |
| 15. TOTAL (sum of lines 13 and 14)                                     |                       | 7705        | 7705              | 7705        | 7705        |
| SECTION 3 - BUDGET ESTIMATES OF NEED FOR FUNDING NEEDS FOR THE PROJECT |                       |             |                   |             |             |
| (a) Grant Program                                                      | (b) First             | (c) Second  | (d) Third         | (e) Fourth  |             |
| 16.                                                                    | \$ 29405              | \$ 29405    | \$                | \$          |             |
| 17.                                                                    |                       |             |                   |             |             |
| 18.                                                                    |                       |             |                   |             |             |
| 19.                                                                    |                       |             |                   |             |             |
| 20. TOTAL (sum of lines 16-19)                                         | \$ 29405              | \$ 29405    | \$                | \$          |             |
| SECTION 4 - OTHER BUDGET INFORMATION                                   |                       |             |                   |             |             |
| 21. Direct Charges:                                                    | 22. Indirect Charges: |             |                   |             |             |
| 23. Remarks:                                                           |                       |             |                   |             |             |

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## ASSURANCES - NON-CONSTRUCTION PROGRAMS

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the Office of Management and Budget, Paperwork Reduction Project (0348-0040), Washington, DC 20503.

**PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.**

**NOTE:** Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.
2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.
3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.
4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.
5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).
6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee 3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.
7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.
8. Will comply, as applicable, with provisions of the Hatch Act (5 U.S.C. §§1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 18 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.
10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is \$10,000 or more.
11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and, (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).
12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.
13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).
14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.
15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.
16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.
17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, "Audits of States, Local Governments, and Non-Profit Organizations."
18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

|                                                                           |                                  |
|---------------------------------------------------------------------------|----------------------------------|
| SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL<br><i>A. J. [Signature]</i>   | TITLE<br><i>4/15/99</i>          |
| APPLICANT ORGANIZATION<br><i>California Department of Fish &amp; Game</i> | DATE SUBMITTED<br><i>4/16/99</i> |

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